

SURVEY AND IDENTIFICATION OF TOSPOVIRUS BUD NECROSIS

ISOLATES INFECTING TOMATO *LYCOPERSICON*

ESCLENTUM MILL. IN ANDHRA PRADESH

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ABSTRACT

Tomato samples showing the symptoms of bud necrosis were collected from farmers' fields in the major tomato growing areas of Chittoor, Kurnool, Kadapa, Guntur and Ananthapur districts of Andhra Pradesh. The virus causing bud necrosis on tomato was maintained on cowpea Cv.C-152 by sap inoculation and confirmed as groundnut bud necrosis virus (GBNV) of tomato isolate by DAC-ELISA.

KEYWORDS: Tomato, Groundnut Bud Necrosis Virus (GBNV), DAC-ELISA

Received: Jul 13, 2016; **Accepted:** Aug 06, 2016; **Published:** Aug 11, 2016; **Paper Id.:** IJASRAUG201635

INTRODUCTION

In Andhra Pradesh, tomato is grown very extensively in Chittoor district followed by Kurnool, Kadapa, Guntur, Ananthapur, Nizamabad, Prakasam, Krishna, Khammam, and East & West Godavari. Major markets for tomato export are located at Madanapalli and Palamaneru in Chittoor district and Aluru, Aspari, Pyapili and Pattikonda in Kurnool district.

Being a Solanaceous crop, tomato is subjected to the attack of many plant pathogens such as fungi, bacteria and viruses which cause serious losses to the crop yields. Of which, the diseases caused by viruses are of major importance. About 36 viruses are reported to occur on tomato all over the world. Among the viruses, tospoviruses are the most destructive ones and reported to cause about 60 per cent yield loss. The most important tospo virus infecting tomato include tomato spotted wilt virus (TSWV) in USA, Spain, Taiwan and Argentina and peanut bud necrosis virus (PBNV) in India. PBNV seems to be endemic in India and its host range indicates that legumes and other hosts play a major role in disease occurrence (Ghanekar *et al.*, 1979a; Singh and Krishna Reddy, 1996). The occurrence of tospo viruses has been reported on several crops like chilli, brinjal, cowpea, potato, clustersbean, mungbean, urdbean, muskmelon, soybean, peanut from different places in India.

GBNV-To, a member of the genus *Tospovirus* of the family Bunyaviridae, is the most economically important virus of peanuts in the Indian subcontinent which is transmitted by melon thrips, *Thrips palmi* in a propagative manner (Reddy *et al.*, 1992). GBNV-To disease caused by tospovirus was reported on groundnut as early as 1949 (Reddy *et al.*, 1995). The first record of the virus with symptoms similar to that of tospoviruses was seen in the Annual Report of the Indian Agricultural Research Institute in 1949. Tomato spotted wilt virus is one of the most destructive viruses limiting tomato production world wide (Rosello *et al.*, 1995). It was 1st reported in

Australia in 1915. In India, the occurrence of TSWV on tomato (Prasada Rao *et al.*, 1980) and several other crop plants. TSWV based on symptoms was observed for the first time on Marglobe tomato variety in 1964 from Niligiris in Tamil Nadu. The characteristic symptoms of TSWV are necrosis of the young growing bud, bronzing of the leaves with brown necrotic lesions, followed by wilting of the plants in severe cases. The ripened fruits exhibit circular markings as concentric bands of red and yellow broken rings about 1cm in diameter (Todd *et al.*, 1975).

MATERIALS AND METHODS

Collection, Isolation and Maintenance of Virus Isolates

Samples were collected from major tomato growing areas of Chittoor, Kurnool, Kadapa, Ananthapur, and Guntur, East and West Godavari and Nizamabad districts of Andhra Pradesh. Thirty nine GBNV infected tomato samples were collected from tomato fields. Infected tomato plant samples showing the symptoms of chlorotic and necrotic lesions on leaves, bronzing on stem, ring spots on fruits were collected at the vegetative, flowering and fruiting stages of the crop. These samples were brought to the laboratory in air tight polythene bags in ice buckets and kept in refrigerator at 4°C for further studies.

MECHANICAL INOCULATION

Preparation of Potassium Phosphate Buffer

Potassium phosphate buffer at 0.05M and pH 7.0 was prepared in the laboratory with the following reagents for extraction and isolation of virus from the infected samples.

Dipotassium hydrogen phosphate (K_2HPO_4) - 5.4 g, Potassium di phosphate (KH_2PO_4) - 2.4 g, β - Mercaptoethanol - 1.56ml, Distilled water - 1000ml

Preparation of Inoculum

The infected tomato plant samples collected from farmers' fields were washed thoroughly in running tap water to remove dirt and then blotted dry. The inoculum was prepared (1:10 w/v) by macerating the infected leaves in the presence of pre-chilled 0.05 M potassium phosphate buffer (pH 7.0) containing 2-mercaptoethanol. The entire process of grinding was done under chilled conditions. Apply the inoculum immediately onto the tested plants.

Method of Inoculation

Well established, young actively growing tomato and cowpea plants raised in insect proof cages in greenhouse were used for virus inoculation. Inoculations were done on fully expanded primary leaves on test plants. Prior to inoculation, a pinch of carborundum/ celite was dusted over the selected leaves. A sterile cotton pad immersed in the inoculum was gently rubbed over the leaves thrice in one direction. Care was taken not to damage the leaves during inoculation. Immediately after the inoculation, the leaves were washed off with a jet of sterile distilled water to remove the excess of inoculum and abrasive. The inoculated plants were labeled and kept under observation in insect proof cages in glasshouse for symptom expression.

The same procedure was adopted for isolation of virus from all the 39 tomato samples collected from farmers' fields.

MAINTENANCE OF VIRUS ISOLATES

Virus isolates were maintained on cowpea cv. C-152, since cowpea has consistently produced more local lesions within 4 to 5 days after inoculation.

DAC – ELISA

The direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) as described by Hobbs *et al.* (1987) was adapted for detection and confirmation of the presence of groundnut bud necrosis virus (GBNV) in all test plants. Prior to performance of ELISA, the following solutions are freshly prepared and kept ready in refrigerator at 4°C for use. For direct antigen coating ELISA one gram of the virus infected fresh leaf tissue was extracted in carbonate buffer pH 9.6 containing 0.01 M DIECA (1/10) and purified virus was diluted with carbonate buffer to 1/1000 dilution.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

At present, the detection and diagnosis of the *tospoviruses* and plant viruses in general, is mainly relevant on serologically based procedures. For many years, the lack of quality tospovirus specific antisera made serological tests impractical (Francki and Hatta, 1981). Eventually, high quality polyclonal antisera were produced (Gonsalvens and Trujillo, 1986) and ELISA now has been adopted as the main means of detecting and diagnosing *tospoviruses* (Resende *et al.*, 1991) and variants used for *tospovirus*-specific monoclonal antibodies (Sherwood *et al.*, 1989; Huguenot *et al.*, 1990; Adam *et al.*, 1991). ELISA has also been adopted to allow the detection of TSWV within individual thrips (Cho *et al.*, 1988; Bandla *et al.*, 1994), demonstrating both the sensitivity and versatility of the technique.

Hobbs *et al.* (1987) standardized direct antigen coating (DAC) and protein A coating (DAC) forms of indirect ELISA and compared them with the double-antibody sandwich (DAS) form of ELISA for detection of PBNV (it was referred as TSWV). Louro (1994) evaluated different ELISAs for the detection of TSWV in plant extracts. Among the different types of ELISAs viz., IEM, Biotin mediated ELISA (which is more sensitive than the standard ELISA), dot immunobinding assays and tissue immunoassays are commonly used for detection. It is suggested that tissue-print immunoassay is simple, rapid and an inexpensive method for testing samples in a large or small scale which could be useful for diagnosis in quarantine cases and for plant healthy certification programme.

PROCEDURE FOR DAC-ELISA

Programme sheet was prepared according to the number of samples used in the test. Antigen samples were prepared in carbonate buffer and 0.1 ml was added to each well of the microtitre plate (Falcon) as shown in the programme sheet and the plate was incubated for 2 hrs at 37 °C. The plate was washed thrice with PBS-T by keeping 3 min interval each time. Antiserum was diluted with PBS-TPO from 1: 100 to 1: 10,000 and was added to the corresponding wells (0.1 ml/well) as shown in the programme sheet. The plate was covered with lid and incubated at 37°C for 2 hrs. The plate was washed 3 times with PBS-T. The goat antirabbit antibodies labeled with ALP was diluted to 1:10000 with PBS-TPO and added to the plate. The plate was incubated at 37°C for 2 hrs and washed with PBS –T for 3 times. The enzyme substrate P-nitrophenyl phosphate was added to the wells and incubated at room temperature for 30 min. The reaction was terminated by adding 50 µl of 3 N NaOH solutions to each well. The reaction of the each sample was noted according to yellow color intensity

RESULTS AND DISCUSSIONS

Collection, Isolation and Maintenance of Virus Isolates

Thirty nine tomato samples showing the symptoms of chlorotic and necrotic lesions on leaves, bronzing on stem, ring spots on fruits were collected from farmers' fields in the major tomato growing areas of Chittoor, Kurnool, Kadapa, Ananthapur, Guntur, Godavari and Nizamabad districts of Andhra Pradesh (Plate 1,2,3). The virus was maintained on cowpea cv. C-152 in the laboratory. Cowpea was selected as an assay host for the maintenance of the virus, since it has consistently produced more local lesions within 4 to 5 days after inoculation (Plate 4). The identity of the virus isolates were further confirmed by DAC-ELISA. Twelve samples have shown positive reaction under DAC- ELISA (Plate 5), and the OD values were recorded at 450 nm from the infected and healthy plants (Table 1). Out of twelve isolates which have shown positive reaction, six isolates representing one each from Kurnool, Kadapa, Chittoor, Guntur, Nizamabad and Godavari were selected for further studies. These results were in agreement with that of the following findings.

Watermelon bud necrosis *tospovirus* was successfully transmitted by mechanical sap inoculation technique using 0.02 M 2-mercaptoethanol in 0.1 M Potassium phosphate buffer pH 7.0 and found cowpea cv C-152 as a good assay host (Krupashankar *et al.*, 1998).

Hemalatha (1999) transmitted the tomato necrotic tospo virus by mechanical inoculation using 0.5 M potassium phosphate buffer pH 7.0 containing 0.02 M 2-mercaptoethanol and found cowpea cv. C-152, *Datura stramonium* and *Chenopodium amaranticolor* as good assay hosts.

Ho Xuan Thien *et al.* (2002) studied the natural infection of tospo virus on mungbean. Symptomatic mungbean samples reacted positively with groundnut bud necrosis virus and watermelon silver mottle virus antisera in DAC-ELISA. The virus was maintained in cowpea which served as a propagation host. The polyclonal antiserum against N protein with the nucleocapsid protein of the virus homologous titer 1:512 could detect tospo virus isolates from cowpea, potato and tomato collected from different locations.

Ho xuan thien *et al.* (2003) reported that *Tospovirus* infection on mungbean (up to 70 per cent) was noticed at Indian Agricultural Research Institute experimental farm, New Delhi in different varietal trials. Symptoms under field conditions included necrosis of plant parts such as leaves, stems, petioles, growing points, buds and pods, Symptomatic mungbean plants showed positive reaction with groundnut bud necrosis virus (GBNV) and watermelon silver mottle virus (WSMV) antisera in direct antigen-coated enzyme-linked immunosorbent assay. *Tospovirus* was easily transmitted to different plant species belonging to families Fabaceae, Cucurbitaceae, and Solanaceae. Bhat *et al.* (2001) reported that a survey for the presence of *tospovirus* infections on blackgram, cowpea, greengram, and soybean fields at Delhi during August to October 1999 was carried out and veinal necrosis, tip necrosis, stem, petiole and pod necrosis, and chlorotic lesions were the common symptoms observed in affected plants. Isolates induced chlorotic and necrotic lesions, and systematic infection in cowpea (cv. Pusa Komal) upon mechanical inoculation. Of the seven different tospovirus antisera tested against these isolates indirect antigen-coated (DAC-ELISA), positive reactions were seen only with groundnut bud necrosis virus (GBNV) and watermelon silver mottle virus (WSMV). In nucleic acid hybridization tests, isolates hybridized only with nucleocapsid protein (NP) gene of GBNV and not with NP gene of tomato spotted wilt virus (TSWV). Non-reaction of soybean isolates with WSMV antiserum indicated antigenic variation among the isolates.

CONCLUSIONS

The tospovirus virus causing bud necrosis on tomato was maintained on cowpea by sap inoculation and the identity of the virus isolates were confirmed as groundnut bud necrosis virus (GBNV) of tomato isolate by DAC-ELISA.

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Table 1: O. D. At 450 Nm Indirect-Antigen Coated Enzyme Linked Immunosorbent Assay

Virus Isolate	Absorbency at 450 nm	Healthy	
		H1	H2
VI 1	0.55	0.159	0.062
VI 2	0.99		
VI 3	0.101		
VI 4	0.060		
VI 5	0.52		
VI 6	0.72		
VI 7	0.55 +Ve C		
VI 8	0.54 +Ve KNL		
VI 9	0.73 +Ve KNL		
VI 10	0.65 +Ve KAD		
VI 11	0.045		
VI 12	0.44		
VI 13	0.139 +Ve G		
VI 14	0.347 +Ve C		
VI 15	0.51		
VI 16	0.44		
VI 17	0.63		
VI 18	0.50 +Ve E & W		
VI 19	0.46		
VI 20	0.48 +Ve N		
VI 21	0.52		
VI 22	0.48		
VI 23	0.44		
VI 24	0.139		
VI 25	0.347		
VI 26	0.49		
VI 27	0.51 +Ve KAD		
VI 28	0.44 +Ve G		
VI 29	0.63		
VI 30	0.050		
VI 31	0.046		
VI 32	0.46		
VI 33	0.45		
VI 34	0.40		
VI 35	0.41		
VI 36	0.42		
VI 37	0.37		
VI 38	0.41		
VI 39	0.48		

O.D. Optical density, VI = Virus isolate, C=Chittoor, Kad= Kadapa, G=Guntur, E& W= East and West Godavari, KNL=Kurnool.



**Plate 1: Tomato Leaves Showing Necrotic Spots Collected from Kurnool
DT. Fields for Preparation of Standard Inoculum**



Plate 2: Tomato Plants Stem Showing Necrosis/Bronzing Color Symptoms



Plate 3: Tomato Fruit Showing Ring Spots Caused By GBNV – To



Plate 4: Symptoms of GBNV-to on Cowpea C-152 by Mechanical Sap Inoculation

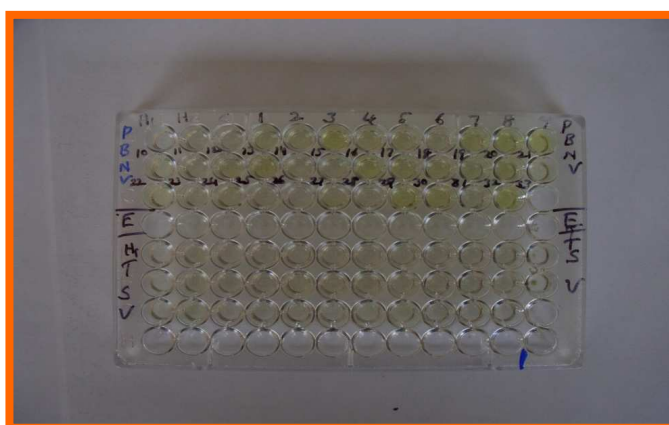


Plate 5: Symptoms Produced By GBNV – To – Positive in DAC-ELISA on Cowpea Cv-152